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COMPARISON OF BREAKING STRENGTH AND SHELL THICKNESS AS EVALUATORS OF WHITE-FACED IBIS EGGSHELL QUALITY

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Abstract—Data from a 1986 field study of white-faced ibis (*Plegadis chihi*) nesting at Carson Lake, Nevada, were used to compare the utility of eggshell strength measurement and eggshell thickness as indicators of eggshell quality. The ibis population had a history of reproductive failure correlated with elevated egg concentrations of *p,p′*-DDE, hereafter referred to as DDE. Eggs from 80 nests (one egg/nest) were tested for shell strength and thickness. Egg contents were analyzed for organochlorines, mercury and selenium; productivity at each nest (minus one egg) was monitored in the field. DDE-DDT concentrations in the eggs ranged from none detected (<0.1) to 29 ppm (wet weight). Shell thickness and shell strength were both negatively correlated with DDE (−0.60, −0.61, respectively), but shell strength deteriorated at a faster rate than shell thickness. Scanning electron micrographs indicated the deterioration in strength was related to changes in ultrastructure as well as to decreased thickness. Fourteen eggs with <0.40 ppm DDE were used to exemplify normal "control" eggs. Of the eggs with higher concentrations of DDE (i.e., ≥0.40 ppm), 11 of 66 were thinner (>2 SD below "control" mean) than normal, 11 of 59 were weaker than normal and 7 eggs were cracked so their strength could not be tested, although thickness was measured. Therefore, 17% of the eggs with ≥0.40 ppm DDE were thinner than normal and 27% were either weaker than normal or cracked. Further, six eggs (four with ≥15 ppm DDE) did not have abnormally thin shells, but did have abnormally weak shells. Nests with abnormal test eggs (thinner, weaker or cracked) produced fewer young than nests with normal eggs. Use of the shell strength parameter provides additional information for better evaluations of reproductive problems. The potential utility of monitoring eggshell quality goes beyond evaluating effects of organochlorines since recent work indicates that other environmental hazards can affect shell quality.

Keywords—White-faced ibis Eggshell thickness Eggshell strength Eggshell ultrastructure DDE

INTRODUCTION

There has been concern about negative effects of environmental contaminants on avian reproduction since Ratcliffe [1] recognized that DDE thinned peregrine falcon (*Falco peregrinus*) and European sparrowhawk (*Accipiter nisus*) eggshells. Relatively low concentrations of DDE have been correlated with eggshell thinning, eggshell cracking and reduced reproductive success in many species throughout the world [2].

Monitoring for contaminant hazards that contribute to avian reproductive failure is an ongoing concern [3]. Recent research has identified continuing DDE-associated shell thinning and reduced productivity for black-crowned night-herons (*Nycticorax nycticorax*) inhabiting the intermountain western United States [4] and for bald eagles (*Haliaeetus leucocephalus*) inhabiting Oregon's lower Columbia River region [5]. It has been also demonstrated that some organophosphorus compounds can have temporary effects on shell quality. Parathion reduced Coturnix quail (*Coturnix coturnix*) eggshell thickness [6] and methyl parathion reduced northern bobwhite (*Colinus virginianus*) shell thickness, strength and weight (J.K. Bennett, unpublished data). Eggshell thinning also has been observed in the Eurasian dipper (*Cinclus*

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cinclus) inhabiting acidified streams [7]. Finally, the U.S. Environmental Protection Agency (EPA) [8] frequently tests chemicals for avian reproduction hazards and requires shell thickness measurements as part of the pesticide registration process. Therefore, measuring eggshell quality remains a valuable tool for testing or monitoring a variety of contemporary environmental hazards.

The standard procedure for evaluating eggshells of wild birds is usually either a direct measure of eggshell thickness or a thickness index (shell weight/egg length × egg width) [1] compared to controls or eggs collected before the DDT era (pre-1947). Several researchers have encouraged measuring both shell strength and thickness to improve evaluations of shell quality. Cooke [9] showed a linear relationship between the shell thickness index and breaking strength for peregrine falcon, European sparrowhawk and gray heron (*Ardea cinerea*), but strength decreased at a greater rate than thickness. Carlisle et al. [10] suggested that an egg's resistance to breakage and penetration may be more directly related to the integrity of the egg than to shell thickness. Mechanisms other than thinning, such as porosity or poor mineralization, could contribute to weakness. Their tests demonstrated that, under laboratory conditions, mallard (*Anas platyrhynchos*) shell strength was significantly reduced at a lower dietary DDE concentration than was shell thickness. Bennett et al. [11] compared the shell quality of eggs of bobwhites fed sulfanilamide and demonstrated that shell strength measurements identified more abnormal eggs than shell thickness. They also demonstrated reduced shell strength and abnormal shell ultrastructure without a reduction in shell thickness. Therefore, the three studies led to the conclusion that breaking strength can be a more sensitive indicator of abnormal eggshells than shell thickness.

In 1985 and 1986, a nesting study of white-faced ibis (*Plegadis chihi*) was conducted at Carson Lake near Fallon, Nevada [12]. Eggs from the population were heavily contaminated with DDE. Eggs contained up to 29 ppm DDE wet weight and cracked and dented eggs were common. Therefore, this ibis population provided an opportunity to compare eggshell breaking strength and thickness measurements as indicators of contaminant effects on the shell quality of wild birds. Intact eggs collected in 1986 were tested for breaking strength and eggshell thickness, and they were examined by scanning electron microscopy (SEM). In this paper, we present results of the technique comparisons.

METHODS

One egg was randomly collected from each of 80 white-faced ibis nests. The mean clutch size was 3.34 eggs before an egg was collected. All eggs were freshly laid or had little incubation (<4 d). Subsequent nest productivity was monitored by nest observation [12]. The eggs were examined upon collection for cracks; 7 of the 80 eggs were cracked prior to collection.

Shell strength was measured with an Instron universal testing instrument (Model 1130) using published methods [11]. Eggs were compressed at the equator between two parallel stainless steel surfaces advancing at a constant rate of 0.4 mm/min with a 2-kg maximum load range. A chart recorder advancing at 100 cm/min recorded the compression on the egg and the load (±1%) at which the egg failed (broke). Typical shell failures were either a small hairline fracture or a cratering at the place on the egg where the load had been applied.

Shell thickness (including membranes) was measured at three sites on the egg equator with a micrometer graduated in units of 0.01 mm; the average of the three measurements was used to represent the thickness of the shell. Egg contents were analyzed for organochlorine pesticides and their metabolites at the Patuxent Wildlife Research Center with a 0.1-ppm (wet weight) detection limit [12].

SEM was used to describe the ultrastructure of selected eggshells and to examine them for contaminant-associated abnormalities using methods described by several workers [11,13–15]. Twelve eggshells representing the range of strength and thickness measurements were selected for SEM examination. A shell fragment of approximately 1 mm^2 was removed from the equator region of the dried shell, mounted on an aluminum stub and gold sputtered. Photographs of shell cross sections at 250 × magnification allowed examination of the shell membrane attachments, mammillae and palisade layer.

An analysis of the reproductive performance of this ibis population [12] concluded that mercury, selenium and organochlorines other than DDE were not correlated with the reproductive parameters studied. Therefore, this study examined only the relationship of shell quality and levels of DDE contamination. Simple linear regression was used to examine the relationship between shell quality and DDE residues, and the relative response of shell thickness and shell strength. For a further comparison of shell quality measurements with DDE concentrations, the eggs were divided into six categories of DDE contamination (<0.40, 0.40–1.0, 1.01–4.0, 4.01–8.0, 8.01–16.0 and >16.0 ppm DDE), similar to the categories established in 1985 [16] for white-faced ibis and used by Henny and Herron [12] in other analyses of these ibis data. In this study, the <0.40-ppm category was included to define the shell characteristic of the least-contaminated eggs to serve as normal "control" values. One-factor analysis of variance (ANOVA) was used to examine the mean shell quality measurements for each DDE category. In tests where ANOVA were significant ($p \leq 0.05$), Bonferroni means comparison was used to separate means.

RESULTS AND DISCUSSION

Relation between shell strength and thickness

The relationship between DDE-contaminated white-faced ibis shell strength and thickness is shown in Figure 1. The relationship is linear within the measurement range and significant ($p < 0.0001$). Similar highly significant linear relationships between strength (measured by a piercing test) and the thickness index were presented by Cooke [9] for both European sparrowhawks and peregrine falcons. For each species, the starting point was the mean thickness index for all the pre-DDT era shells. Then, based on the regression, Cooke calculated a very similar relationship between percentage decrease in strength (y) and percentage decrease in thickness index (x) for each species. The relationships were:

European sparrowhawk	$y = 1.45x$
Peregrine falcon	$y = 1.54x$
Gray heron	$y = 1.46x$.

The equation from our study, where strength was measured by a compression test and using thickness (mm) instead of thickness index (mg/mm^2), was:

White-faced ibis	$y = 1.98x$.

In Cooke's study, each slope was approximately 1.5; a decrease in shell thickness of x% for any of his species meant a decrease in shell strength of $1.5x$%. With the ibis, based on a compression strength test, shell strength decreased at nearly twice the rate of shell thickness.

The plot of shell strength vs. shell thickness (Fig. 1) also demonstrates the relative change in each measurement at various concentrations of DDE in the eggs. The broken line indicates a theoretical equivalency line between eggshell strength and thickness as measurements decline from the es-

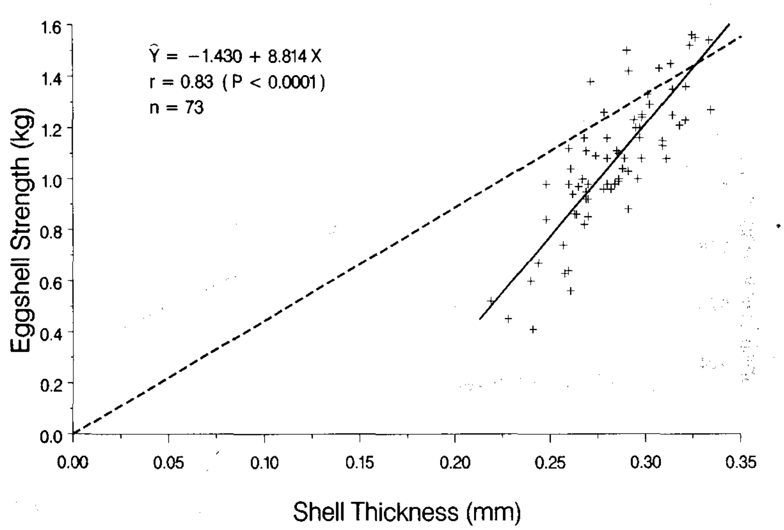

Fig. 1. Relationship between eggshell thickness (mm) and eggshell breaking strength (kg) for white-faced ibis. The dashed line indicates a theoretical equivalency line between eggshell strength and thickness as measurements decline from the estimated normals (0.327 mm thickness and 1.45 kg strength) toward zero for each.

timated normals (0.327 mm thickness and 1.45 kg strength, as described below) toward zero for each. If the two methods detected an equivalent reduction in shell quality, the plot of the measurements should approximate the equivalency line. Because most of the values were below the line, and all the highly contaminated eggs were well below the line, we concluded that the two methods were not equivalent in describing differences in shell quality. There was a greater reduction in shell strength than in thickness in this series of DDE-contaminated eggs. Similar analyses, yielding the same conclusion, resulted from tests with European sparrowhawk and peregrine falcon eggs [9] and northern bobwhite eggs [11].

The strong linear correlation between strength and thickness suggests that both parameters would be correlated to a similar degree with log DDE. Indeed, shell thickness ($\hat{Y} = 0.288 - 0.025 \log_{10}$ DDE, $r = -0.60$, $n = 80$) and strength ($\hat{Y} = 1.127 - 0.250 \log_{10}$ DDE, $r = -0.61$, $n = 73$) were similarly correlated (Figs. 2 and 3).

Identification of abnormal eggs

For further examination of this data set, eggs with the least contamination (14 eggs with <0.40 ppm DDE) were used to define the normal "control" range for each of the shell measurements (thickness $\bar{x}$, 0.295 mm, SD, 0.023; and strength $\bar{x}$, 1.21 kg, SD, 0.19). In this study, abnormal eggs were defined as those with strength and thickness measurements that were >2 SD below the mean of eggs with <0.40 ppm DDE.

It must be pointed out that the <0.40-ppm group included eggs that contained small, but measurable, amounts of DDE. Average white-faced ibis shell thickness was 0.327 mm prior to the DDT era [17] and, based on that thickness and the regression equation in Figure 1, we estimated normal shell strength at 1.45 kg. Figure 1 indicates that only a few eggs sampled in this study had that thickness or strength. Two eggs without detectable DDE (<0.10 ppm) had shell thickness of 0.318 and 0.314 and strength readings of 1.21 and 1.25 kg, respectively. Therefore, our ability to identify abnormal eggs was biased when slightly contaminated eggs (<0.40 ppm DDE) were used as "controls." However, the bias applies to both data sets and should not confound the objective of this study, i.e., to compare the relative utility of the two parameters to identify abnormal eggs.

The ANOVA and comparisons of thickness and strength means produced similar results (Table 1). In both cases, significant reductions from the normal values were detected in eggs with >8 ppm DDE. Note that the sample sizes were smaller in the shell strength categories because seven cracked eggs were not tested for strength. These results differ from Carlisle et al. [10], who found that strength measurements detected significant differences in mallard eggs at lower dietary concentrations of DDE than shell thickness. However,

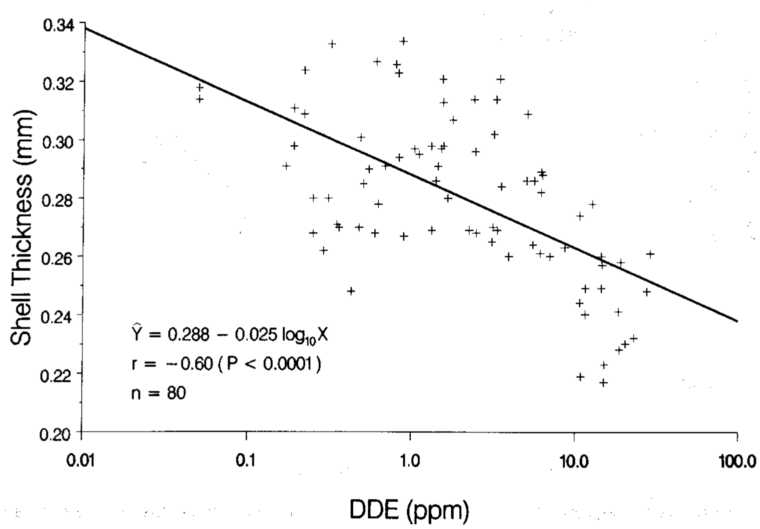

Fig. 2. Relationship between log DDE (ppm, wet weight) and eggshell thickness (mm) for white-faced ibis.

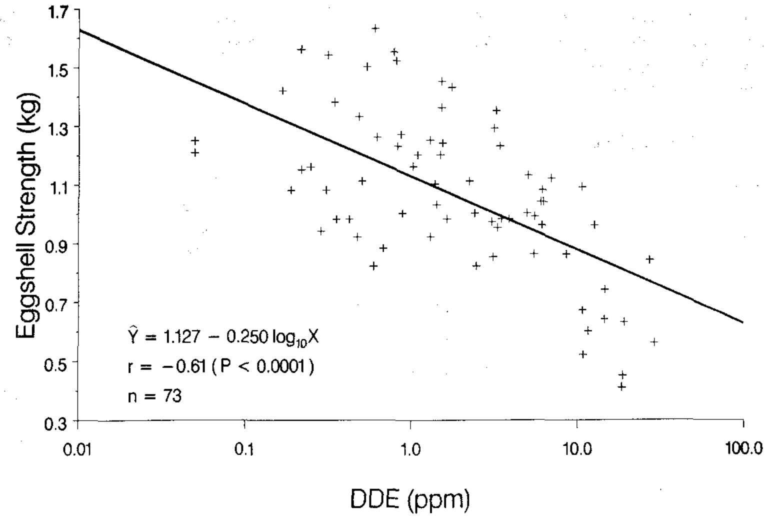

Fig. 3. Relationship between log DDE (ppm, wet weight) and eggshell breaking strength (kg) for white-faced ibis.

Carlisle's dietary concentrations of DDE were discrete categories of treatment, rather than the continuous concentration categories used in this study, which probably increased their ability to identify DDE effects.

The percent of normal values (Table 1) indicate that the measured mean differences in shell strength (up to 52% difference) were much greater than the amount of difference measured in shell thickness (up to 18% difference), as illustrated in Figures 2 and 3. The number of eggs with abnormal (>2 SD below "control" mean) shell characteristics and the relative amounts of DDE found in the eggs are shown in Table 2. Thickness identified 11 abnormal eggs (17%) out of the 66 more contaminated eggs (≥0.4 ppm DDE). Strength tests could be conducted on only 59 of those eggs, yet 11 were identified as abnormally weak (19%). Most importantly, of the 11 eggs identified as weak, 6 were not abnormally thin. Therefore, the strength test identified six more eggs (10% of those sampled) as abnormal than did thickness measurements. The characteristics of those six eggs are presented in Table 3. All of those eggs had measurable amounts of DDE, and most were highly contaminated (≥15 ppm DDE). These findings agree with laboratory results

Table 1. White-faced ibis eggshell measurements relative to egg DDE concentrations (ppm wet weight)

	Shell thickness				Shell strength			
DDE (ppm) concentration	Mean (mm)	SD	*n*	% Normal[a]	Mean (kg)	SD	*n*	% Normal[a]
<0.40	0.295A[b]	0.023	14	100	1.21A[b]	0.19	14	100
0.40–1.0	0.293A	0.026	14	99	1.21A	0.27	14	100
1.01–4.0	0.291A	0.019	24	99	1.12A	0.18	23	93
4.01–8.0	0.281A	0.016	9	95	1.02A	0.08	9	84
8.01–16.0	0.248B	0.020	12	84	0.76B	0.20	8	63
>16.0	0.243B	0.013	7	82	0.58B	0.17	5	48
			80				73	

[a]Normality is based on eggs containing <0.40 ppm DDE.
[b]Column means with different capital letters are significantly ($p \leq 0.05$) different.

Table 2. White-faced ibis eggs with cracks, abnormal thickness and abnormal strength relative to egg DDE concentrations (ppm wet weight)

DDE (ppm) concentration	n	No. of cracked eggs	Abnormal thickness[a]	Abnormal strength[a,b]
<0.40	14	0	–	–
0.40–1.0	14	0	1	1
1.01–4.0	24	1	0	1 (of 23)
4.01–8.0	9	0	0	0
8.01–16.0	12	4	5	5 (of 8)
>16.0	7	2	5	4 (of 5)
Total	80	7	11	11 (of 59)

[a]Abnormal eggs are those with shell thickness less than 0.249 mm (i.e., "control" mean − 2 SD); shell strength less than 0.830 kg.
[b]Strength measurements of seven cracked eggs not included here, but presented in Table 4.

Table 4. Characteristics of white-faced ibis eggs that were cracked prior to collection

Thickness (mm)	Measured strength (kg)[a]	Predicted strength (kg)[b]	DDE concentration (ppm wet weight)
0.314	1.08	1.34	2.4
0.249	0.73	0.76	12
0.249	ND	0.76	15
0.232	0.56	0.61	23
0.230	ND	0.59	21
0.223	0.40	0.53	15
0.217	ND	0.48	15

ND, not determined.
[a]Strength test conducted only on eggs with small dents; eggs with large cracks not tested.
[b]Based on the model: $\hat{Y} = -1.430 + 8.814X$, where X = shell thickness.

where the strength test detected more low quality shells than thickness measurements [10,11].

Approximately 9% of the eggs sampled in this study were cracked prior to random collection and they were among the thinnest-shelled eggs in the series. We examined the possibility of obtaining useful strength measurements on four of the seven cracked eggs. The four eggs had only small dents and were tested like the intact eggs, but were oriented so the load was not applied directly to the dents. These cracked eggs produced a compression profile similar to intact eggs. The measured strength of the four eggs with minor dents was similar to the predicted strength (Table 4), but this study does not provide sufficient information to recommend using strength measurements from cracked eggs.

Table 3. DDE concentrations (ppm wet weight) and shell characteristics of white-faced ibis eggs with abnormal strength (<0.830 kg) but normal thickness (≥0.249 mm)

DDE (ppm) concentration	Strength (kg)	Thickness (mm)
0.60	0.82	0.268
2.5	0.82	0.268
15	0.64	0.260
15	0.74	0.257
19	0.63	0.258
29	0.56	0.261

Abnormal eggs and reproductive success

Productivity at the nests (number young/nest) was also related to shell characteristics. Shell thickness identified 11 abnormal eggs and the production at those nests was 43% of nests with normal thickness (Table 5). Breaking strength also identified 11 abnormal eggs and production at those nests was 63% of nests with normal strength. Production at nests that had test eggs with normal thickness but abnormal strength was about 68% of normal strength and 70% of normal thickness. Nests with cracked test eggs also had low production. Therefore, the information provided by all these examinations was biologically meaningful because reproduction was reduced in nests with cracked or abnormal eggs. However, 17% of the eggs with ≥0.40 ppm DDE were thinner than normal, whereas 27% were either weaker than normal or cracked.

Shell ultrastructure

An SEM photograph of an ibis egg with a shell thickness of 0.324 mm, which is very similar to the pre-DDT era mean of 0.327 mm, is shown in Figure 4. The breaking strength was 1.56 kg, and the egg contained only 0.22 ppm DDE and no detectable DDT. The shell ultrastructure exhibits distinct mammillae, which form the foundation of the shell, and a thick palisades layer, which contributes to the shell thickness, all characteristics of normal shell ultrastructure [15,18]. We speculate that this egg is representative or nearly representative of a pre-DDT era white-faced ibis egg. Its ultrastructure

Table 5. Young white-faced ibis produced per nesting attempt from eggs with different shell characteristics

Category	Young produced 0	1	2	3	n^a	Young/attempt
Thickness (mm)						
≥0.249 (normal)	10	13	34	11	68	1.68
<0.249 (abnormal)	6	2	3	0	11	0.73
Total	16	15	37	11	79	1.54
Strength (kg)						
≥0.830 (normal)	8	11	32	10	61	1.72
<0.830 (abnormal)	3	4	4	0	11	1.09
Total	11	15	36	10	72	1.63
Cracked	5	0	1	1	7	0.71
Abnormal strength but normal thickness[b]	1	3	2	0	6	1.17

[a]Complete reproductive data obtained for 79 of 80 nests (one nest not relocated).
[b]Young produced from the six nests with eggs having shell strength <0.830 kg but thickness ≥ 0.249 mm.

in cross section appears similar to that described for the domestic chicken (*Gallus domesticus*) [19], Coturnix quail [15] and northern bobwhite [11].

An ibis egg with abnormal shell quality is shown in Figure 5. The eggshell thickness was 0.228 mm (30% below pre-DDT norm), its breaking strength was 0.45 kg (69% below pre-DDT norm) and the egg contained 19 ppm DDE and 1.6 ppm DDT. The eggshell was not only much thinner than the shell shown in Figure 4, but it also had irregularly shaped mammillae, which contribute to shell weakness [11,15].

CONCLUSIONS

This study, based on eggs from a wild population of birds, demonstrated that shell strength identified more contaminant-associated abnormalities than shell thickness. Shell strength decreased more than shell thickness for specific DDE concentrations, a phenomenon first described by Cooke

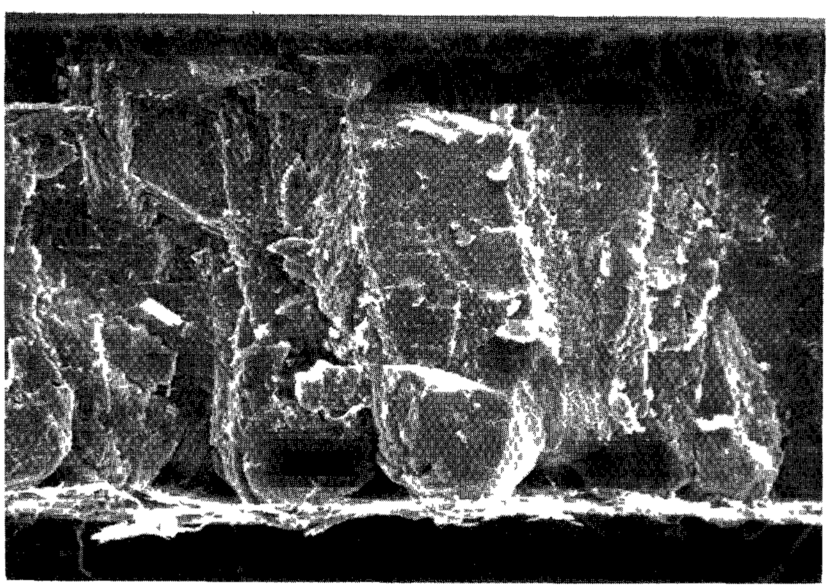

Fig. 4. SEM photograph (250×) of a good quality white-faced ibis eggshell. This shell exhibits distinct, regularly spaced mammillae on the bottom shell layer and a thick palisades layer above.

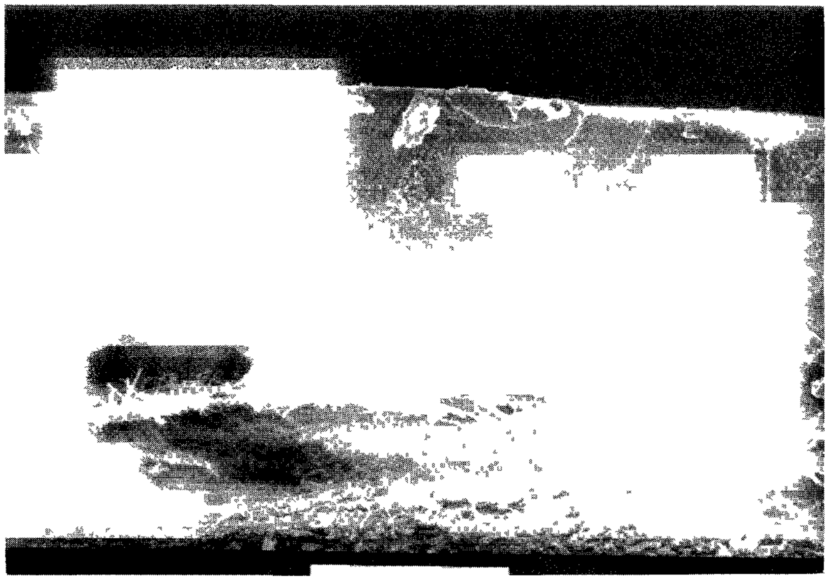

Fig. 5. SEM photograph (250×) of a poor quality white-faced ibis eggshell. This shell has few and irregularly shaped mammillae and a thin palisades layer.

[9]. The deterioration in strength apparently was related to abnormal shell ultrastructure as well as to thinner shells. These data were part of a field study where reduced reproduction was related to reduced eggshell thickness, reduced eggshell strength and egg breakage. We conclude that eggshell strength measurements may provide additional information for better evaluations of potential reproductive problems under field conditions. Therefore, strength measurements are useful in both the laboratory and in the field. But strength measurements may not always be practical for avian field studies because of problems transporting intact potentially fragile eggs to a testing instrument. The type of instruments used to determine eggshell strength are usually available at universities with mechanical engineering, food science or materials testing departments.

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